IMMUNOLOGY AND DISEASE RESISTANCE LABORATORY

Brief Laboratory Review

May 9, 1996

LIVESTOCK AND POULTRY SCIENCES INSTITUTE
AGRICULTURAL RESEARCH SERVICE
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BELTSVILLE, MARYLAND

1996
Brief Review

Immunology and Disease Resistance Laboratory
Livestock and Poultry Sciences Institute

May 9, 1996

APU Conference House, Building 1050, BARC-East

I. AGENDA

8:15 AM	Refreshments	
8:30 AM	Opening Remarks	T. J. Sexton, ID, LPSI
8:45 AM	Program and Administrative Issues	J. K. Lunney, RL, IDRL
9:15 AM	Research Highlights	A. Guidry M. Paape H. Lillehoj M. Jenkins R. Fayer
10:15 AM	Break	
10:30 AM	Research Highlights (con't)	J. Urban J. Lunney D. Zarlenga M. Fleming L. Gasbarre
11:45 AM	Break	
12:00 PM	Executive Session	
12:45 PM	Joint Lunch of Review Team with IDRL SY's	

II. PROGRAM ISSUES

1. Current Mission Statement

The mission of the Immunology and Disease Resistance Laboratory is to improve the safety and health of farm animals and consumers by protecting animals and the environment against parasitic infections and mastitis. The goals of the MU are to evaluate the immunobiology of the host response to parasitic and mastitis infections; protect consumers from zoonotic diseases in food or water caused by parasitic diseases; assess cytokines and other immune effectors for prevention and control of parasitism and mastitis in farm animals; determine immune and genetic factors that control responses of livestock and poultry to parasitic diseases and mastitis, and the genes the encode such host disease resistance associated factors; map farm animal genomes to identify candidate genes with useful immunologic properties or with effects on parasitic disease resistance; define mechanisms through which the parasite or bacterium modifies host physiology and controls pathogen induced stress, and identify and develop methods to control parasite problems that decrease sustainability of American agriculture.

2. Research Accomplishments (1993-1995)

Mastitis

A panel of antibiotic residue screening tests currently used for milk from individual cows and goats have been independently screened and shown to identify milk that was free of antibiotics. False positive results from some screening tests in farm bulk tank milk had resulted in the dumping of milk. Comparative studies proved that certain tests can be used with cow and goat milk without undue economic losses to dairymen. Use of these screening tests will provide a more healthy product to the consumer.

Developed methods to quantitate a variety of bovine neutrophil cell surface receptors and their expression. Monoclonal antibodies were produced which enhanced phagocytosis and killing of mastitis pathogens by bovine neutrophils. These reagents, developed for the prevention and treatment of mastitis, will also reduce antibiotic contamination of the human food chain. Research resulting from use of these reagents will play a pivotal role in establishing the pathophysiology of mastitis, in tailoring vaccination strategies and production of immunotherapeutic reagents.

A newly developed ELISA and western blotting technique revealed the presence of nitrotyrosine residues in milk from inflamed udders, which resulted in tissue damage and lost milk production, and accounted for 60% of the two million dollar annual loss due to mastitis. The discovery of nitrotyrosine residues in milk will lead to development of strategies to neutralize their tissue damaging effects.

Mastitis increases during the periparturient period when depressed serum calcium is prevalent. Ionic calcium was shown to play an important role in neutrophil phagocytosis, and binding of IgG_2 and IgM opsonic antibodies to neutrophils increased cytosolic free Ca^{2+} concentrations which are important in signaling initiation of many neutrophil responses. Providing adequate calcium and opsonic antibodies during the periparturient period should lead to more effective neutrophil phagocytosis and prevention of mastitis.

Developed mammary teat, duct and secretory epithelial cell models that include the epithelial cells subcellular matrix, fibroblast and endothelial cells that simulates the blood/milk barrier in the mammary gland. These models were used to determine factors affecting the passage of neutrophils and antibiotic passage through the blood/milk barrier, changes in neurotphil function following diapedesis, effectiveness of antibiotics against bacteria internalized in epithelial cells, etc. Based on results with this mammary epithelial cell model, a dramatic difference among cows in the ability of their neutrophils to diapedesis across the blood/milk barrier was shown. This could be used in selecting cows that are resistant to infection.

Cryptosporidiosis

Of numerous chemical disinfectants tested against *Cryptosporidium parvum* oocysts few have shown activity. Though bleach (Clorox) was reported useful for disinfection of surfaces in hospital settings we found oocysts infectious after 2 hr in 100% bleach. In a study to determine the disinfecting ability of small molecular weight gases, after 24 hr exposure of an aqueous suspension of oocysts to ammonia, formaldehyde, ethylene oxide, bromomethane or ozone no oocysts remained infectious. Oocysts also were killed when held at temperatures of 64°C for 5 min or 72.4°C for 1 min or at -10, -15 and -20°C for >1 week, >24 hr, and >8 hr, respectively, indicating that temperatures below boiling render water safe to drink but ice made with contaminated water remains infectious for hours or days. These careful laboratory tests will enable consumers to treat water so that *Cryptosporidium parvum* oocysts are killed, thus preventing cryptosporidial infections.

At the request of the American Water Works Association and the USDA, a 20-minute video tape was produced, explaining the biology, transmission, prevention and treatment of *Cryptosporidium*. Over 1000 copies have been distributed to water treatment plant staff, public health workers, agriculturalists, veterinarians and state, federal and international government administrators.

Although white-tailed deer are the most prevalent wild ruminant in North America no studies had examined them for *Cryptosporidium parvum* infection. In a cooperative study we determined that does and fawns spontaneously became infected with *Cryptosporidium parvum* and therefore might serve as reservoirs of infection for other animals. Other wildlife including fish, reptiles and amphibians could not be artificially infected with *Cryptosporidium parvum*, contrary to a previous report. These results will help scientists identify sources and plan new strategies to decrease *Cryptosporidium parvum* contamination of the water supply.

Pioneering immunological studies were conducted which demonstrated that exogenous interleukin-12 (IL-12), a cytokine that stimulates T cell development to the T helper 1 (Th1) subtype, followed by the ability to express interferon-gamma (IFN-γ), completely protected neonatal mice from experimental infection with *Cryptosporidium parvum*. These studies will set the stage for development of novel biopharmaceuticals to control this waterborne infection.

Groundbreaking studies identified a neutralizable surface protein of *Cryptosporidium parvum*, we cloned the gene for this protein, expressed it, and immunized cows with the recombinant protein, or jet-injected sheep with a construct of this gene, stimulating both to produce high antibody levels in serum and colostrum; the gene was licensed to private industry.

Neosporosis

Identified and characterized native antigens of *Neospora caninum* that are useful for diagnosis of neosporosis in domestic and companion animals. Developed the first recombinant antigens from *Neospora* for diagnosing acute neosporosis in cattle. The DNA sequences encoding these antigens are being patented and, after application is made, the recombinant *Neospora* proteins will be licensed to a biotechnology company for development of a diagnostic test kit for neosporosis in cattle and domestic animals.

Chicken Coccidiosis

Developed a method for attenuating three different *Eimeria* parasites with gamma-irradiation for immunizing chickens against coccidiosis without causing clinical signs of disease. This work is now being expanded to floor pen trials to reproduce field conditions in the poultry industry. Adapted the gamma-irradiation method for investigating genes and gene products that may serve as targets for protective immunity and for incorporating into antigen delivery vectors. Using genetic engineering methods, produced a recombinant *Eimeria* sporozoite antigen in the yeast *Pichia pastoris* and used this antigen to immunize chickens against coccidiosis. Large-scale purification of the yeast-derived antigen is underway in preparation for floor pen vaccination trials.

Due to increasing drug-resistance problem with coccidiosis, immunity-based control strategies are required. Immunohistological studies showed that sporozoites preferentially invade intestinal CD8 T cells. Based on this observation, we developed stable chicken hybridomas which secrete monoclonal antibodies which will block parasite invasion of these lymphocytes. These results are important in developing novel immunologic control of coccidiosis in chickens.

Swine Food Safety

Exogenous recombinant murine IL-4 cures established gastrointestinal nematode infections, while IL-4 antagonists exacerbate disease. The development of DNA probes, and competitive molecular assays, for porcine IL-4, and a variety of other Th1 and Th2 related cytokines (IL-2, IL-10, and IL-12), as well as production of specific antibodies against these cytokines, will define swine responses to economically important extracellular and intracellular parasites. IL-4 has immune system dependent and independent activities, and is capable of enhancing intestinal smooth muscle cell contraction and epithelial cell secretion. There is potential for development of porcine analogs as natural physiological and immunological modifiers of resistance to parasite infections.

A direct link between infection of swine with the *Trichuris suis* and enhanced bacterial-induced necrotic proliferative colitis was shown. The invading bacteria were identified as *Campylobacter*-like species which potentially threaten carcass quality and food safety. Anthelmintic drug and vaccine prophylaxis are effective in reducing the propensity for secondary bacterial disease.

Led the First International Swine CD Workshop (and Cochaired the Second Workshop) that resulted in the characterization and international standardization of a broad panel of monoclonal antibodies which define swine lymphoid cell markers. Veterinary and biomedical researchers need these monoclonal antibodies to assess normal swine immunity and to probe disease and vaccine responses. For *Toxoplasma gondii* infections these monoclonal antibodies have identified an important regulatory cell subset, the CD4+/CD8+ cell, that is increased early in the infection.

Developed a genetically engineered serology test for bovine and swine cysticercosis. At present, the FSIS visually inspects beef products for the larval stage of a parasite that, when ingested, causes tapeworm infections in humans. This inspection procedure is no longer adequate and misses up to 35% of infected animals. We developed a test based upon genetically engineered parasite proteins that is useful in the serodiagnosis of infection prior to the meat products reaching the consumer. When fully developed, this test will assist inspectors in providing safer meat products to American consumers. Patents are pending on this development.

Determined potential for non-synchronic infections of freeze-resistant and domestic species of *Trichinella* within the same host. At present, *Trichinella* parasite in infected meat can only be inactivated by freezing or cooking; however, there exists in nature a freeze-resistant species of *Trichinella*. Contrary to current scientific evidence, we have demonstrated the capability for the two putative species of *Trichinella* to non-synchronically infect the same host and thus potentiate gene flow between species and introduce the freeze-resistant genotype into the domestic cycle. Results from this work may directly affect recommendations on the safe storage of uncooked pork products.

Cattle Gastrointestinal Nematodes

Developed procedures and reagents to assess lymphoid population and cytokine changes in the local lymphoid tissues of cattle infected with *Ostertagia ostertagi*. Showed that *Ostertagia* induces a marked increase in lymphocyte numbers in the draining lymph nodes and the mucosa of the abomasum. This lymphoid expansion favors B cells and T cells positive for the $\gamma\delta$ T cell receptor, with a subsequent diminution of classic $\alpha\beta$ T cell receptor positive T lymphocytes. Developed reagents and methodology to thoroughly investigate the transcription of genes encoding the cytokines IL-2, IL-4, IL-10, IL-12 and IFN-γ as well as of control genes (HPRT and β-actin). Infection favors transcription of IL-4 and IL-10, and decreased transcription on IL-2 and perhaps IFN-γ. This pattern of cytokine response is considered to be the classic protective immune response to intestinal nematode infections, yet is apparently ineffective for *Ostertagia*. This discrepancy is an area of current research emphasis.

Experimental protocols were defined that demonstrated enhanced circulating cortisol in cattle and sheep throughout gastrointestinal nematode infections when compared to pre- and post-infections levels. Adrenal responsiveness to ACTH challenge also was enhanced during infections.

Bovine immune response mechanisms to zoonotic parasites such as *Cryptosporidium parvum* and to worms which affect the well being and food producing capabilities of the animal are not well known. We identified, cloned and expressed, in experimental quantities, the bovine cytokine IL-12 and IL-15 genes which are believed to play important roles in regulating the animals' capability to immunologically respond to parasite infections. This work has allowed us to study the capability of these cytokines to function both prophylactically and indirectly as adjuvants in attenuating bovine infections resulting from parasites as well as other pathogens.

Developed DNA probes and PCR assays for the diagnosis of pathogenic parasitic infections of cattle. Cattle harbor numerous types of parasitic infections; however, only certain types are pathogenic to their hosts and require expensive drug treatment. We have developed PCR based methods as well as repetitive DNA probes to rapidly differentiate and quantify the numerous types of parasites within the host. The application of these techniques by diagnostic laboratories will permit veterinarians to avoid costly and time consuming drug treatments for animals infected with nonpathogenic parasites.

Initiated program to assess potential problems posed by gastrointestinal nematode infections in intensive rotational grazing systems. As a part of this program, developed in conjunction with grazing and pasture scientists, a questionnaire that has been distributed throughout the northeastern US. The purpose of the questionnaire is to assess the impact on parasite-induced production losses of switching to intensive rotational grazing systems. This questionnaire has since been requested by a number of grazing groups and research scientists for use in the analysis of other grazing programs.

Genetics of Disease Resistance

Demonstrated that parasite gastrointestinal nematode fecal egg counts in cattle are characterized by an "overdispersed" distribution; only a small number of cattle remain highly susceptible to infection, and are responsible for the majority of parasite transmission, and thus should be the target of control procedures. Determined that fecal egg counts (EPG) in cattle are influenced by host genetics (heritability = 0.2-0.3) as is the ability to produce anti-parasite antibody responses (heritability = 0.8-0.9). The latter, however, do not correlate with fecal egg values indicating that although both are under genetic control, the resulting values are influenced by different genes or sets of genes.

Produced a small herd (40-45 reproductive females) of bovine major histocompatibility complex (BoLA) identical animals. Bulls with demonstrated propensity for producing high and low EPG calves were then used to produce parasite "resistant" and "susceptible" lines. Measured phenotypic traits that are useful as indicators of parasite numbers (level of resistance): fecal EPG for *Cooperia* sp., and serum pepsinogen, shifts in gut T cell populations, and cytokine profiles for *Ostertagia*. These cattle are now being used to define the immunological mechanisms which render cattle immune or susceptible to infection with gastrointestinal nematodes.

Lack of information concerning host immunogenetics in avian coccidiosis impedes breeding of disease resistant chickens. Basic immunogenetic studies demonstrated a relationship between host genetics and the efficacy of recombinant antigen immunization. Immunological studies demonstrated that tumor-necrosis factor and gamma-interferon production differs in genetically defined chickens which show different levels of coccidia disease susceptibility. These results enhance our understanding of genetic control of immune response to coccidia and will be useful for chicken breeding program for poultry industry.

A panel of new molecular markers on swine chromosome 6 has been developed using flow sorted and microdissected chromosome 6. In pigs, traditional breeding attempts to increase lean meat have resulted in selecting swine in certain breeds which carry the defective allele for porcine stress syndrome. The new panel of chromosome 6 markers should help identify genes associated with carcass quality traits and clearly distinguish them from the gene that encodes the porcine stress syndrome defect. Use of such markers should enable scientists to move forward with marker assisted selection studies for pigs with improved carcass traits.

Identified swine which are genetically resistant to *Trichinella spiralis* infections. At least two genes encode this disease resistance, one of which has been mapped to the swine leukocyte antigen complex. Extensive immune analyses have yet to identify the mechanism for this resistance. More extensive mapping studies are planned to help breeders identify genetically resistant stock. These results will help identify novel mechanisms for controlling foodborne parasitic diseases.

3. **Productivity Summary** (Publications for 1993-1995)

Scientist	1st Author	Co-Author	Senior Author Credit	Popular Articles	Abstracts
Fayer	11	11	13	0	5
Fleming	8	0	8	0	5
Gasbarre	4	5	6	2	12
Guidry	3	15	11	0	13
Jenkins*	7	11	10	0	10
Lillehoj*	20	13	29	0	19
Lunney	9	24	14	2	14
Paape	5	32	28	3	29
Urban	5	15	8	2	14
Zarlenga*	9	8	12	2	21
TOTALS	81	134	139	11	142

*Patent(s) Submitted or Awarded

4. CRIS Summary

CRIS Number	Title	Net-to-Location	Expires
1265-31320-012	Identification and Mapping of Genes Involved in Parasitic Disease Resistance/Susceptibility	$555,838	9-30-00
1265-32000-044	Immunobiology of Ostertagia and Other Gastrointestinal Nematodes of Cattle	$623,914	12-6-98
1265-32000-048	Reduce Mastitis Incidence and Antibiotic Use by Enhancing Cow's Natural Defenses	$721,155	3-31-00
1265-32000-049	Strategies to Control Swine Parasites Affecting Food Safety	$708,064	9-30-00
1265-32000-050	Prevention and Therapy for Protozoan Parasites Affecting Food Animals, Food Safety, Public Health	$872,398	10-1-00

III. ADMINISTRATIVE ISSUES

A. Summary of Financial Resources

Resource	FY95* Actual	FY96 Actual	FY97 Projected	FY98 Projected
Net-to-Location	$3,525,342	$3,449,077	$3,449,077	$3,449,077
Temp. Water Qual. Funds	25,000	175,000	125,000	125,000
Indirect Research Cost	999,412	1,019,818	1,155,902	1,302,146
Other Fixed Costs	681,157	654,468	674,102	694,325
Adjustments				
Temporary funds	17,025	6,858		
Spec. coop. agreement	6,500	40,000		
Net to MU	1,846,248	1,902,933	1,744,073	1,577,606
Salary	1,412,100	1,476,078	1,520,360	1,565,971
All Other	434,148	426,855	223,713	11,635
Salary: All Other	3.25	3.46	6.79	134.59
Number of SYs	9.23	9.5	9.5	9.5
Bench dollars/SY	384,652	381,481	376,219	376,219
Discretionary dollars/SY	47,037	44,932	23,549	1,225
Percent discretionary	12.2%	11.7%	6.2%	0.03%
Percent fixed costs	87.8%	88.3%	93.8%	99.97%

*Funding calculated from PIL FY95 funding and MSML funding for mastitis CRIS. Outside funding for FY95 $348,000; Outside funding for FY96 $175,750.

B. Managerial Problems

1. Budget

The MU has addressed problems in funding through several approaches. Scientists have worked with NPS and industry to identify areas for potential new funding initiatives. Such an initiative was developed for FY97 for chicken coccidiosis but was unfortunately not submitted with the ARS budget information. The MU has initiated projects in areas defined by ARS as high priority as outlined below in Responses to Previous Reviews. It is hoped that as funds are redirected to high priority research areas that IDRL projects will be designated as fund recipients. Budget realities have already forced the unit to decrease its staff by not filling vacancies, or by reassigning personnel in, support positions and by changing a support scientist position into a lower GS level temporary position. In addition, laboratory and animal facilities have been greatly reduced, as outlined in Facilities below, to the point that newly refurbished buildings have had to be vacated and service contracts on sophisticated equipment terminated.

Scientists in IDRL have been extremely active in attracting outside support. In FY96 four USDA CSREES NRI grants were submitted of which 2 were funded. External support from industry and other sources has expanded our research program; several IDRL scientists exchanged visits with relevant companies and their staff to identify new funding sources. However, these commercial interactions are usually not long-term. In FY95, three industry funding opportunities were withdrawn due to company buyouts, termination of trials due to negative results, or the company's inability to generate sufficient biotechnology funding.

2. Facilities

Each scientist in the MU has reassessed laboratory and animal facilities usage. This has resulted in a 10% decrease in overall animal space at the same time that shared space is added as animal efforts are integrated with other LPSI units for outbred swine production and for chicken hatching operations. Yet disease studies require separate controlled animal facilities which, to meet current animal care standards, require expensive upgrading. The MU has made major efforts to set up collaborative research arrangements so that laboratory space and equipment is used more efficiently within the MU and between the MU and other laboratories in LPSI. For FY97 the scientists in B.173 have completely reorganized their laboratory space, resulting in an almost 30% decrease in overall space usage.

Attempts have been made to improve the existing facilities. In FY95, the Area Office contributed over $100,000 to support some of these needs (roof replacement and repair of laboratory areas and painting and flooring repairs in animal facilities). These contributions are appreciated and it is hoped that this level of support for facilities maintenance will be continued. The long range plans for improvement of all research facilities, as outlined in the LPSI Modernization Program, should address most long-term needs for improved facilities.

C. Safety and Health Report

The BA Safety, Occupational Health and Environmental Section held a walk-through of the Laboratory on March 6, 1995 with only minor problems noted for correction. A memo sent from the Laboratory to SOHES on April 7, 1995 documented corrective action taken on problems noted in the inspection report. Regular inspections at bimonthly intervals are performed by the IDRL Safety Officer.

Within the last year, the Laboratory has taken several steps to improve the safety of Laboratory operations. These include: 1) reduction in radioisotope usage by conversion of assays from radioactive to fluorescent and other dye based assays; 2) proactive monitoring of laboratory personnel and animal caretakers for exposure to *Toxoplasma gondii* infections; and 3) installation of eyewash stations in all animal facilities.

D. Response to Recommendations From Previous Reviews

Following in depth lab reviews in five LPSI laboratories in 1992-1994 a series of management reorganizations were proposed to address future program needs based on recommendations made by the external review teams, NPS and BA and LPSI management. In May 1994 the Parasite Immunobiology Laboratory (PIL), the predecessor of IDRL, was formed from the reorganization of the four Animal Parasitology Unit laboratories. The PIL, along with its new sister units, the Parasite Biology and Epidemiology Laboratory (PBEL) and the Biosystematics and National Parasite Collection Unit (BNPCU), were directed to 1) reduce program scope by prioritizing research issues and areas; 2) build multidisciplinary teams around strategic plans for solving important problems; and 3) design a new organizational structure built around these program needs.

In response to this directive, PIL scientists organized a series of strategic planning sessions which resulted in a set of completely reorganized research priorities: 1) enhancing cryptosporidiosis research efforts; 2) emphasizing research in swine parasites associated with food safety; 3) adding sustainable agriculture initiatives and stress assessment in cattle parasitology studies; 4) targeting chicken coccidiosis studies to defining drug-free control mechanisms; and 5) adding a new initiative to define genes which control disease resistance. Three newly written CRIS projects (1265-31320-012, 1265-32000-049, and 1265-32000-050) were the final product of this process.

In October 1994 the Milk Secretion and Mastitis Laboratory (MSML) had its in depth lab review. Following reviewers' recommendations scientists in that laboratory were similarly encouraged to reassess research priorities. Several new CRIS projects were developed during that process, including the mastitis CRIS 1265-32000-048 which emphasized novel in vitro methods of assessing bacterial interactions with host immune cells and mammary tissue. The Immunology and Disease Resistance Laboratory was established in October, 1995, and combined the research areas of two scientists from the

Milk Secretion and Mastitis Laboratory with those of the former Parasite Immunobiology Laboratory in order to build a larger unit whose research was focused on host responses against infectious diseases. The detailed IDRL mission statement is given on p.2 of this booklet.

When the Milk Secretion and Mastitis Laboratory was abolished, the part of the mastitis CRIS project that focused on genetic control of mastitis was shifted to the Gene Evaluation and Mapping Laboratory's (GEML) CRIS project (1265-31000-061) on mapping genes associated with somatic cell scores. The SY associated with that effort, Dr. R. Miller, has since retired. To continue adding mastitis disease information for that mapping effort GEML and IDRL had planned to initiate a joint project in FY97 by assigning 10% of Dr. M. Paape's effort to that CRIS. However, the president's FY97 budget proposal has eliminated one of GEML's CRIS projects (1265-31000-054); this will significantly alter GEML's financial flexibility and thus their abiility to expand their mastitis research efforts.

IV. RESEARCH HIGHLIGHTS

The Immunology and Disease Resistance Laboratory has five CRIS projects with the following objectives:

1265-31320-012, Identification and Mapping of Genes Involved in Parasitic Disease Resistance/Suceptibility (Lunney, Gasbarre, Lillehoj)

Objectives: 1) Identify and breed livestock and poultry that are genetically resistance to parasite infections; 2) Define response phenotypes of parasite resistant vs susceptible animals; 3) Assess role of candidate genes in encoding parasitic disease resistance/susceptibility; 4) Use flow sorted and microdissected chromosomes to develop new swine chromosome specific genetic markers; 5) Use species genome maps to begin to correlate genotype markers with phenotypic traits associated with parasitic disease resistance/susceptibility.

Anticipated Outcomes: Parasite resistant and susceptible individuals will be identified for chicken coccidiosis, for swine trichinosis and toxoplasmosis, and for cattle gastrointestinal nematode infections. Comparison of disease resistant and susceptible individuals will reveal the details of the protective immune mechanisms against these infections thus enabling scientists to design novel anti-parasite control strategies that utilize naturally occurring as well as vaccine induced immunity. As genetic markers for resistant or susceptible individuals are developed producers will be able to use this information in breeding programs to enhance disease resistance and vaccination thus decreasing dependence on drug usage and reducing residues in food and the environment. Identification of parasite susceptible individuals will enable producers to target appropriate treatments including drug therapy and vaccination to susceptible stock as part of an integrated pest management system that would reduce costs and contribute to a more sustainable system of food animal production.

1265-32000-044, Immunobiology of Ostertagia ostertagi and Other Gastrointestinal Nematodes of Cattle (Gasbarre, Zarlenga, Fleming)

Objectives: 1) Characterize cellular immune responses in resistant and non-resistant animals, with special emphasis on the identification and development of new methods and reagents to study cytokine gene transcription, and the use of bovine cytokines as adjuvants or prophylactic agents in bovine enteric infections; 2) Identify parasite antigens and parasitic stages that induce or modulate protective immunity in cattle; 3) Identify phenotypic and genotypic markers that correlate with resistance or enhanced susceptibility to gastrointestinal nematode infection; 4) Define immunoregulatory factors and their relationships to endocrine stress factors, associated with *O. ostertagi* infection; 5) Development management strategies for enhancing the immunological responsiveness and the overall health and well-being of cattle exposed to gastrointestinal nematode infections.

Anticipated outcomes: Current control of gastrointestinal nematodes in cattle is predicated on the repeated use of broad spectrum anthelmintics. While these drugs are very effective, a number of factors make it imperative that additional means of parasite control be available. Factors include the widespread appearance of multiple drug resistance in gastrointestinal nematodes species, changing societal attitudes towards drug residues in food and the environment, and the development of more intensive grazing programs. Increased knowledge of the basis for immunity to the parasites, and the development of management programs that maximize naturally or vaccine induced immunity will be important in the development of integrated pest management programs that would lessen producer costs, and contribute to more sustainable systems of meat and dairy production. The development of reagents to identify and produce bovine cytokines and of methods to delineate the bovine immune system has already proven to be important to scientists working in a broad range of infectious diseases of cattle, including studies of viral, bacterial, and protozoal pathogens throughout the US. Finally the definition of stressor effects of gastrointestinal nematode infections will provide information necessary to alleviate the postulated immunosuppressive effects of such infections, allowing for the more effective use of immunotherapy such as vaccinations to control bovine infectious diseases, and thus enhancing the overall health and well-being of American cattle.

1265-32000-048, Reduce Mastitis Incidence and Antibiotic Use by Enhancing Cow's Natural Defenses (Guidry, Paape)

Objectives: 1) Enhance role of phagocytic cells in preventing mastitis; 2) Enhance immunity against mastitis patogens; 3) Characterize differences in mastitis resistance of daughters of high and low milk-somatic-cell-count sires; 4) Characterize cell surface antigens recognized by anti-bovine neutrophil monoclonal antibodies; 5) Production of bispecific antibodies; 6) Clone bovine complement receptors; 7) Characterize nitrotyrosine mediated mammary tissue damage during mammary inflammation; and 8) Role of C5a and tumor necrosis factor on neutrophil phagocytosis and oxidative burst.

Anticipated Outcomes: Development of bispecific antibodies will produce an alternative to antibiotics for mastitis therapy and have broad application in resistance to other diseases in animals and humans. Will provide monoclonal antibodies and other reagents for studying functionally important cell surface receptors on neutrophils. Will develop strategies to neutralize the tissue damaging effects of nitrotyrosine residues. Enhancement of neutrophil function through use of immunomodulators will prevent mastitis.

1265-32000-049, Strategies to Control Swine Parasites Affecting Food Safety (Urban, Lunney, Zarlenga, Hill)

Objectives: Reduce transmission of foodborne pathogens of swine: 1) Define cytokine-regulated immune mechanisms that protect pigs against parasites that threaten food safety; 2) Study parasite proteases and other secreted products as regulators of host immunity and pathology; and 3) Identify DNA sequences for diagnosis and for expression of specific and shared antigens for a broad spectrum anti-worm vaccine.

Anticipated Outcomes: Technologies developed to measure specific antibody secreting cells by ELISPOT and cytokine expression by RT-PCR and ELISPOT will help define stereotypical immune responses to the intracellular protozoans, *Toxoplasma gondii* and *Cryptosporidium parvum* versus the extracellular helminths, *Trichuris suis* and *Trichinella spiralis*. Immunogenicity of the native thiol and metalloproteases from *T. suis* will be defined and expressed recombinant molecules will be tested as protective immunogens or for development of specific inhibitors that block worm physiology. DNA probes will identify potentially zoonotic strains of *T. spiralis* that are resistant to standard freezing procedures and present a threat to consumers. Cross reactive antigens shared by several economically important helminth parasite are being identified that, in combination with specific antigens and through the use of novel vaccine delivery techniques, will be developed into a broad spectrum anti-helminth vaccine; the potential use of a DNA vaccine will also be considered. These outcomes will provide a rationale basis for diagnosis and induction of appropriate immunity to important zoonotic parasite infections in swine using the most modern immunological and molecular strategies.

1265-32000-050, Prevention and Therapy for Protozoan Parasites Affecting Food Animals, Food Safety and Public Health (Fayer, Jenkins, Lillehoj, Urban)

Objectives: Prevent illness in food animals, control food contamination and protect public health from protozoan parasites, *Cryptosporidium parvum* affecting all mammals and *Eimeria* spp affecting chickens will be targets of immunity based strategies including stimulating gut immunity, enhancing cell mediated immunity, lymphokine mediated immunomodulation, vaccination with DNA, recombinant antigens and attenuated parasites.

Anticipated Outcomes: In-depth understanding of the nature of host-parasite interactions which lead to protection will enable the development of rational immunological control strategies against avian coccidiosis. Cytokines and lymphokines which exert anti-parasitic effect will be used against coccidia instead of drugs thus reducing chemical contamination in food chains. A practical, cost-effective vaccination method using gamma-irradiated coccidian oocysts will be developed to immunize chickens against coccidiosis. Genes and gene products associated with metabolizing intracellular parasites will be incorporated in recombinant vaccines or as targets of chemotherapy. Optimal delivery systems (e.g. folwpox virus, *Salmonella*, DNA gene gun) for vaccination of chickens with recombinant *Eimeria* antigens will be identified.

Development of a quantitative polymerase chain reaction test will result in a faster, more accurate and less expensive test for determining the presence and relative quantity of *Cryptosporidium* in water, soil, manure, and animal tissues than is presently available. Immunological studies will elucidate the cells and cell products that participate in a protective response, enabling scientists to develop strategies to enhance immunization and prevent severe clinical infections. Epidemiologic studies with wildlife and wildfowl will identify potential sources and mechanisms of spread of *Cryptosporidium* in the environment and watershed. Studies with physical and chemical disinfectants will provide new products and techniques for rendering food, water, utensils and surfaces free of infectious organisms. Environmental studies of soils, runoff conditions, and composting of manure will identify methods to prevent contamination of surface waters with agricultural sources of oocysts.

V. SCIENTISTS PROGRESS SUMMARIES

Ronald Fayer
Supervisory Zoologist

CRIS 1265-32000-050, Prevention and therapy for protozoan parasites affecting food animals , food safety, public health

Progress: Identified drugs effective in prophylaxis against cryptosporidiosis in mouse models and calves. Determined water temperatures at which *Cryptosporidium parvum* (Cp) oocysts were inactivated. Discovered that the cytokine IL-12 stimulated protective immunity against cryptosporidiosis. Identified a neutralizable Cp protein, cloned and expressed the gene, immunized sheep by direct gene injection, and cattle, with recombinant protein, and licensed genes to private industry.

Plans: Cellular and cytokine immune mechanisms against Cp are still incompletely understood: mouse knockout strains will be used to identify essential cells and mechanisms; rBoIL-12 will be expressed and tested in calves. Environmental studies will target sources of waterborne Cp from wildlife and waterfowl, and the role of soil types in reducing oocyst runoff from agricultural lands.

For the temporary $250,000/year in Water Quality funds, $125,000 will be given to the Environmental Chemistry Laboratory in the Natural Resources Institute where jointly planned studies on soil types, crop cover, slope of the land, and rainfall will be analysed for their effects on oocyst recovery and viability. A portion of the remaining funds are needed for salaries for 2 biological laboratory technicians (GS-5); one to continuously provide purified parasites essential for all phases of the research and to support expanded molecular biology studies, the other to process water, soil, and composted manure samples for the presence of viable parasites. Equipment essential for determining the presence and viability of oocysts under environmental conditions must be purchased including constant temperature circulators, phase-contrast microscope, automated densitometer, closed containment rodent cages and racks, water pumps and filters, and numerous supplies. This phase of research on cryptosporidiosis is an expansion of our present studies. It will include collaboration with soil, water, and composting specialists at three locations within ARS, and collaboration with wildlife specialists at the University of Georgia. The latter will provide us with biological specimens from white-tailed deer and wetland mammals, which are potential carriers and disseminators of the parasite.

Outside Funding:

CRADA with Elias Shaheen of the Clorox Corporation on Disinfection of Cryptosporidium Oocysts. 1996-1997.
CRADA with Ravi Mennon of the GalaGen, Inc. on Anti-Cryptosporidium Colostrum Titers Development and Production. 1992-1995.
SCA with J. Fischer of the University of Georgia on Cryptosporidium in White Tail Deer and Wetland Mammals. 1995-1996.

Cooperators:

A. Guidry, ARS, USDA, Beltsville, MD
L. Gasbarre, ARS, USDA, Beltsville, MD
D. Zarlenga, ARS, USDA, Beltsville, MD
D. Shelton, ARS, USDA, Beltsville, MD
L. Sikoria, ARS, USDA, Beltsville, MD
W. Stout, ARS, Pennsylvania State University, State College, PA
J. Harp, ARS, NADC, Ames, IA
B. Blagburn, Auburn Univ., Auburn, AL
M. R. Cranfield, Baltimore Zoo, Baltimore, MD
E. Shaheen, Clorox Corporation , San Francisco, CA
G. Jackson, Food and Drug Agency , Washington, D.C.
R. Mennon, Galagen, Inc. , Minneapolis, MN
T. K. Graczyk, Johns Hopkins Univ., Baltimore, MD
S. J. Upton, Kansas State Univ., Manhatten, KS
W. Gause, Uniformed Services Univ. for Health Sciences, Bethesda, MD
F. Finkelman, Univ. of Cincinnati, Cincinnati, OH
J. Fischer, Univ. of Georgia, Athens, GA
P. O'Donoghue, Univ. Queensland, Australia
D. White, Univ. of Tennessee, Knoxville, TN
C. Chappel, Univ. Texas, Houston, TX

Peer-Reviewed Publications (1993-present):

Granstrom, D.E., Dubey, J.P., Davis, S.W., Fayer, R., Fox, J.K., Poonacha, K.B., Giles, R.C. and Comer, P.F. 1993. Equine protozoal myeloencephalitis: antigen analysis of cultured *Sarcocystis neurona* merozoites. J. Vet. Diagn. Invest. 5:88-90.

Fayer, R. and Ellis, W. 1993. Glycoside antibiotics alone and combined with tetracyclines for prophylaxis of experimental cryptosporidiosis in neonatal BALB/c mice. J. Parasitol. 79:553-558.

Fayer, R. and Ellis, W. 1993. Parommoycin is effective as prophylaxis for cryptosporidiosis in dairy calves. J. Parasitol. 79:771-774.

Jenkins, M.C., Fayer, R., Tilley, M. and Upton, S. 1993. Cloning and expression of a cDNA encoding epitopes shared by 15- and 60- kilodalton proteins of *Cryptosporidium parvum* sporozoites. Infection and Immunity. 61:2377-2382.

Elsasser, T.H., Fayer, R., Rumsey, T.S. and Hartnell, G.F. 1994. Recombinant bovine somatotropin blunts plasma tumor necrosis factor-α, cortisol, and thromboxane-B_2 responses to endotoxin *in vivo*. Endocrinol. 134:1082-1088.

Fayer, R. and Ellis, W. 1994. Efficacia della paromomicina nella profilassi della criptosporidiosi dei vitelli da latte. Rev. Zoot. Vet. 22:17-21.

Barr, S.C., Jamrosz, G.F., Hornbuckle, W.E., Bowman, D.D. and Fayer, R. 1994. Use of paromomycin for treatment of cryptosporidiosis in a cat. J. Amer. Vet. Med. Assoc. 205:1742-1743.

Fayer, R. 1994. Development of a precocious strain of *Cryptosporidium parvum* in neonatal calves. J. Euk. Microbiol. 41:40.

Fayer, R. and Ellis, W. 1994. Qinghaosu (Artemisinin) and derivatives fail to protect neonatal BALB/c mice against *Cryptosporidium parvum* (Cp) infection. J. Euk. Microbiol. 41:41.

Zu, S.X., Li, J.F., Barrett, L.J., Fayer, R., Shu, S.Y., McAuliffe, J.F., Roche, J.K. and Guerrant, R.L. 1994. Seroepidemiologic study of *Cryptosporidum* infection in children from rural communities od Anhui, China and Fortaleza, Brazil. Am. J. Trop. Med. Hyg. 51:1-10.

Fayer, R. 1994. Effect of high temperature on infectivity of *Cryptosporidium parvum* oocysts in water. Appl. Envir. Microbiol. 60:2732-2735.

Fayer, R. 1995. Effect of sodium hypochlrite exposure on infectivity of *Cryptosporidium parvum* oocysts for neonatal BALB/c mice. Appl. Environ. Microbiol. 61:844-846.

Fayer, R., Graczyk, T.K. and Cranfield, M.R. 1995. Multiple heterogenous isolates of *Cryptosporidium serpentis* from captive snakes are not transmissible to neonatal BALB/c mice (*Mus musculus*). J. Parasitol. 81:483-484.

Fayer, R. and Fetterer, R. 1995. Activity of benzimidazoles against cryptosporidiosis in neonatal BALB/c mice. J. Parasitol. 81:794-795.

Weigel, R.M., Dubey, J.P., Siegel, A.M., Hoefling, D., Reymolds, D., Herr, L., Kitron, U.D., Shen, S.K., Thulliez, P., Fayer, R. and Todd, K.S. 1995. Prevalence of antibodies of *Toxoplasma gondii* in swine in Illinois in 1992. J. Am. Vet. Med. Assoc. 206:1747-1751.

Jenkins, M., Kerr, D., Fayer, R. and Wall, R. 1995. Serum and colostrum antibody responses induced by jet-injection of sheep with DNA encoding a *Cryptosporidium parvum* antigen. Vaccine 13:1658-1664.

Jenkins, M.C. and Fayer, R. 1995. Cloning and expression of cDNA encoding an antigenic *Cryptosporidium parvum* protein. Molec. Biochem. Parasitol. 71:149-152.

Graczyk, T.K., Cranfield, M.R. and Fayer, R. 1995. A comparative assessment of direct fluorescence antibody, modified acid-fast-stain, and sucrose flotatin techniques for detection of *Cryptosporidium serpentis* oocysts in snake fecal specimens. J. Zoo Wildl. Med. 26:396-402.

Graczyk, T.K., Cranfield, M.R. and Fayer, R. 1996. Evaluation of commercial enzyme immunmoassay (EIA) and immunofluorescent antibody (IFA) test kits for detection of *Cryptosporidium* oocysts of species other than *Cryptosporidium parvum*. Am. J. Trop. Med. Hyg. 54:1996.

Urban, J.F., Fayer, R., Chen, S.J., Gause, W.C., Gately, M.K. and Finkelman, F.D. 1996. IL-12 protects immunocompetent and immunodeficient neonatal mice against infection with *Cryptosporidium parvum*. J. Immunol. 156:263-268.

Fayer, R. and Nerad, T. Effects of low temperatures on viability of *Cryptosporidium parvum* oocysts. 1996. Appl. Environ. Microbiol. (In Press).

Harp, J.A., Fayer, R., Pesch, B.A. and Jackson, G.J. 1996. Effect of pasteurization on infectivity of *Cryptosporidium parvum* oocysts in water and milk. Appl. Environ. Microbiol. (Submitted).

Graczyk, T.K., Fayer, R. and Cranfield, M.R. 1996. *Cryptosporidium parvum* is not transmissible to fish, amphibians or reptiles. J. Parasitol. (Submitted).

Fayer, R., Fischer, J. R., Sewell, C.T., Kavanaugh, D.M. and Osborn, D.A. 1996. Spontaneous cryptosporidiosis in captive white-tailed deer (*Odocoileus virginianus*). J. Wildl. Dis. (Submitted).

Michael Fleming
Research Physiologist

CRIS 1265-32000-044, Immunobiology of Ostertagi and Other Gastrointestinal Nematodes of Cattle

Progress: Research directed at elucidation of the relationships between endocrine stress response and Ostertagiasis commenced less than one year ago. In both cattle and sheep (Haemonchosis), nematode infections increased peripheral cortisol levels throughout the infections and returned to control levels after removal of the parasites. Endogenous stimulation (ACTH) of cortisol also was elevated in infected hosts, indicating increased adrenal cortical sensitivity during nematode infections.

Plans: Endocrine-immunological interactions represent a dialogue among diverse elements of the hypothalamic-hypophysial-adrenal axis and the cellular and humoral components of the immune system. Research goals include an elucidation of this dialogue by correlating defined immulogical alterations (i.e. cytokines, distinct localized cell populations) during Ostertagiasis and the profiles of cortisol production. In addition, management, breed and age related interactions will be assessed relative to cortisol responsiveness.

Cooperators:

R. C. Rhodes III, University of Rhode Island, Kingston, RI
M. Nippo, University of Rhode Island, Kingston, RI
R. H. Fetterer, PBEL, LPSI, BARC, Beltsville, MD

Peer-reviewed publications (1993-present):

Fleming, M. W. 1993. Acute or chronic administration of prolactin alters ovine infections of *Haemonchus contortus*. Vet. Parasit. 50:109-115.

Fleming, M.W. 1993. Catecholamines during development of the parasitic nematode, *Haemonchus contortus*. Comp. Biochem. Physiol. 104C:333-334.

Fleming, M.W. 1993. Selection for a strain of *Haemonchus contortus* that exhibits periparturient egg rise in sheep. J. Parasit. 79: 399-402.

Fleming, M.W. 1993. Ecdysteroids during development in the ovine parasitic nematode, *Haemonchus contortus*. Comp. Biochem. Physiol. 104B:653-655.

Fleming, M.W. 1996. *Haemonchus contortus*: Effects of exogenous hormones relative to periparturient egg rise. J. Parasit. (Submitted).

Fleming, M.W. 1996. In vitro growth of *Ascaris suum* larvae: Cultivation techniques and endocrine regulation. Invert. Repro. Devel. (Submitted).

Fleming, M.W. 1996. Cortisol as an indicator of stress during parasitic infections of *Haemonchus contortus* in lambs (*Ovis aries*). Comp. Biochem. Physiol. (Submitted).

Fleming, M.W. 1996. Experimental inoculation with *Ostertagia ostertagi* elevates peripheral cortisol levels in dairy calves. J. Vet. Med. (Submitted).

Louis Gasbarre
Microbiologist

CRIS 1265-32000-044, Immunobiology of Ostertagia ostertagi and Other Gastrointestinal Nematodes of Cattle (60%)

Progress: Demonstrated that parasite gastrointestinal nematode transmission can be influenced by a small percentage of cattle in the herd and that there may be an immunological basis for this trait. Developed procedures and reagents to assess immunological changes in the local lymphoid tissues of cattle infected with *Ostertagia ostertagi* and used these procedures to define the immunological responses in cattle with different levels of functional immunity to the parasites. Developed nucleic acid probes for the differentiation of the important genera of gastrointestinal nematodes infecting cattle, and compared these probes to existing procedures for the differentiation of eggs passed in the feces. Developed program for assessment of potential parasite effects in intensive rotational grazing systems.

Plans: Continue to define immunological responses in cattle that become functionally immune to gastrointestinal nematode infections, and compare these "responder" cattle to cattle that are unable to become functionally immune. Expand cytokine analyses to include cytokines involved in effector responses, such as IL-1, IL-6, and TNF. Determine stage(s) of the parasite life cycle that are targets for protective immune mechanisms, and life cycle stages with immunoregulatory effects. Assess under field conditions the ability of *Ostertagia* infections to suppress immune responses to unrelated antigens, such as vaccines, and bacterial or viral pathogens. Examine under production conditions the efficacy of targeted treatment of high EPG cattle in the control of gastrointestinal nematodes and subsequent production losses.

CRIS 1265-32000-012, Identification and Mapping of Genes Involved in Parasitic Disease Resistance/Susceptibility (40%)

Progress: Determined that resistance to gastrointestinal nematode infections in cattle are influenced by host genetics and that the use of certain animals can influence parasite transmission. Developed procedures to evaluate parasitologic and immunologic basis for resistance to gastrointestinal nematode infection in calves produced from selective breeding program. Data from these tests are being used to identify phenotypic and genetic markers for resistance/susceptibility. In collaboration with the USDA, ARS, Meat Animal Research Center at Clay Center, NE, began linkage mapping for genes controlling parasite resistance.

Plans: Continue to produce cattle lines that show different capacities to mount protective immune responses against gastrointestinal nematodes, and use these cattle to: 1) assess the genetic basis for resistance and/or susceptibility to gastrointestinal nematode infections, 2) identify genotypic and phenotypic markers of parasite resistance/susceptibility, and 3) in collaboration with MARC attempt to identify and map bovine genes associated with resistance to gastrointestinal parasites.

Outside Funding:

USDA, NRI Grants with D. Zarlenga and A. Canals on T lymphocyte responses to *Ostertagia* in genetically defined cattle. 1994-1997.
USDA, SARE Competitive Grants Program with W. Stout, S. Fales, L. Lohr, and J. Cropper on control of gastrointestinal nematodes in dairy cattle under intensive rotational grazing management. 1995-1998.
INIA, Postdoctoral Fellowship with S. Almeria to analyze cattle cellular immune responses to gastrointestinal nematodes. 1995-1998.
CRADA with Mallinckrodt Animal Health with D. Zarlenga and H. Hughes on uses of bovine cytokines in controlling infectious diseases. 1996-1999.

Cooperators:

J. C. Williams, Louisiana State Univ., Baton Rouge, LA
T. Klei, Louisiana State Univ., Baton Rouge, LA
E. Leighton, CBAR Group, MD
R. Stone, USDA, ARS, MARC, Clay Center, NE
B. Stromberg, Univ. of Minnesota, St. Paul, MN
D. Bryant, Wye Angus, Univ. of Maryland, College Park, MD
W. Stout, USDA, ARS, University Park, PA
S. Fales, Pennsylvania State Univ., University Park, PA
L. Lohr, Lohr Dairy, Hooversville, PA
J. Cropper, .USDA, ARS, University Park, PA
A. Hammond, USDA, ARS, STARS, Brooksville, FL

Peer-Reviewed Publications (1993-present):

Gasbarre, L.C., Nansen, P., Monrad, J., Gronvold, J., Steffan, P. and Hendriksen, S.A. 1993. Serum anti-trichostrongyle antibody responses of first- and second-grazing season calves. Res. Vet. Sci. 54: 340-344.

Nansen, P., Steffan, P.E., Christensen, C.M., Gasbarre, L.C., Monrad, J., Gronvold, J. and Heñdriksen, S.A. 1993. The effect of experimental Trichostongyle infections of housed young calves on the subsequent course of natural infection on pasture. Int. J. Parasitol. 23:627-638.

Gasbarre, L.C., Leighton, E.A. and Davies, C.J.. 1993. Influence of host genetics upon antibody responses against gastrointestinal nematode infections in cattle. Vet. Parasitol. 46:81-91.

Christensen, C.M., Zarlenga, D.S. and Gasbarre, L.C. 1994. *Ostertagia, Haemonchus, Cooperia, Oesophagostomum*: Construction and characterization of genus specific DNA probes to differentiate important parasites of cattle. Exp. Parasitol. 78:93-100.

Gasbarre, L.C. 1994. *Ostertagia ostertagi*: Changes in lymphoid populations in the local lymphoid tissues after primary or secondary infection. Vet. Parasitol. 55:105-114.

Christensen C.M., Zarlenga, D.S. and Gasbarre, L.C. 1994. Identification of a *Haemonchus placei* specific DNA probe. J. Hel. Soc. Wash. 61:249-252.

Gasbarre, L.C. and Stromberg, B.E. 1994. Worms and Germs: How helminth parasitism suppresses bovine immunity, and the implications for deworming and vaccination. Topics in Vet. Med. Smith Kline Beecham Animal Health. 5:4-16.

Zarlenga, D.S., Canals, A., Aschenbrenner, R. and Gasbarre, L.C. 1995. Enzymatic amplification and molecular cloning of the small and large subunits of bovine interleukin 12. Biochem. Biophys. Acta. 1270:215-217.

Gasbarre, L.C., Leighton, E.A. and Bryant, D. 1995. The reliability of a single fecal egg per gram determination as a measure of individual and herd values for trichostrongyle nematodes of cattle. Am. J. Vet. Res. 57:168-171.

Zarlenga, D.S., Canals, A. and Gasbarre, L.C. 1995. A simple and rapid method for constructing internal standards for competitive PCR. BioTechniques. 19:324-326.

Gasbarre, L.C. 1996. New tool for parasite control? The Profitable Cattleman. (In Press).

Al Guidry
Dairy Scientist

CRIS 1265-32000-048, Reduce Mastitis Incidence and Antibiotic Use by Enhancing Cow's Natural Defenses

Progress: Developed in vitro models of mammary teat, duct and secretory cells that have been used to characterize bacterial adherence to mammary epithelium, cytotoxicity of bacterial toxins to mammary epithelium and factors affecting diapedesis of bovine neutrophils from blood to milk. Demonstrated the effectiveness of antibodies in preventing bacterial adherence and reducing the cytotoxicity of bacterial toxins. Developed a method for incorporating antigens into poly (D,L lactide-co-glycolide) microspheres that produce a sustained immune response with affinity maturation. This affords an effective immunization with a single injection, which will have widespread application in animals and humans.

Plans: Information gained from these studies will help clarify the specific mechanisms involved in pathogen/host interactions necessary so that future studies can be planned to formulate vaccines that will prevent bovine mastitis. Development of timed release biodegradable microspheres that enhance the effectiveness of vaccines will allow for comparative analyses of one shot immunization strategies to immunization plus multiple boosts and should lead to more effective mastitis vaccines.

Outside Funding:

OICD and Spanish government support for research with E. Cifrian on in vitro models of mammary teat, duct and secretory cells.
No CRADAs - Working on a possible MIPS agreement between NABI and the University of Maryland and CRADA with Upjohn.

Cooperators:

I. Mather, Dept. Anim. Sciences, Univ. of Maryland, College Park, MD
C. Burvenich, Univ. of Ghent, Ghent, Belgium,
R. Fayer and M. Jenkins, IDRL, Beltsville, MD
A. Fattom, NABI, Rockville, MD
K. Matthews, Univ. Tenn.
S. Srikumaran, Univ. of NE, Lincolm, NE
A.J. Bramley, Univ. VT
E.M. Lilius, Univ. Turku, Turku, Finland

Peer-Reviewed Publications (1993-present):

Guidry, A.J., Berning, L.M. and Hambleton, C.N. 1993. Opsonization of *Staphylococcus aureus* by bovine immunoglobulin isotypes. J. Dairy Sci. 76:1285-1289.

Cifrian, E., Guidry, A.J., O'Brien, C.N., Nickerson, S.C. and Marquardt, W.W. 1994. Adherence of *Staphylococcus aureus* to cultured mammary epithelial cells. J. Dairy Sci. 77:970-983.

Cifrian, E., Guidry, A.J., O'Brien, C.N., Keys, J.E. and Marquardt, W.W. 1994. Bovine mammary teat and ductal epithelial cell cultures. Am. J. Vet. Res. 55:239-246.

Guidry, A.J., O'Brien, C.N., Oliver, S.P., Dowlen, H.H. and Douglass, L.W. 1994. Effect of whole *Staphylococcus aureus* and mode of immunization on bovine opsonizing antibodies to capsule. J. Dairy Sci. 77:2965-2974.

Burvenich, C., Paape, M.J., Hill, A.J., Guidry, A.J., Miller, R.H., Heyneman, R., Kremer, W.D.J. and Brand, A. 1994. Role of the neutrophil leukocyte in the local and systemic reactions during experimentally induced *E. coli* mastitis in cows immediately after calving. Vet. Quarter. 16:45-50.

Matthews, , K.R., Oliver, S.P., Jayarao, B.M., Guidry, A.J., Erbe, E.F. and Wergin, W.P. 1994. Encapsulation of *Streptococcus*, uberis: influence of storage and cultural conditions. Vet. Microbiolol. 39:361-367.

Cifrian, E., Guidry, A.J., O'Brien, C.N. and Marquardt, W.W. 1995. Effect of alpha-toxin and exopolysaccharide capsule on *Staphylococcus aureus* adherence to cultured teat, ductal and secretory mammary epithelial cells. Res. Vet. Med. 58:20-25.

Burvenich, C., Guidry, A.J. and Paape, M.J. 1995. Natural defence mechanisms of the lactating and dry mammary gland. Proc. Intl. Dairy Fed. Sem. Session I:3-13. Tel Aviv, Israel.

Paape, M.J., Capuca, A.V., Guidry, A.J. and Burvenich, C. 1995. Morphology, function and adaptation of mammary cells in normal and disease states. J. Animal Sci. 73 (Supplement 2):1-17.

Burvenich, C., Guidry, A.J. and Paape, M.J. 1995. Natural defence mechanisms of the bovine mammary gland at parturition and drying off. Congresso de Zootecnia In: Revista Portuguesa de Zootecnia. p 48-60.

Cifrian, E., Guidry, A.J. and Marquardt, W.W. 1996. Role of milk fractions, serum and divalent cations in protection of mammary epithelial cells of cows against damage by *Staphylococcus aureus* toxins. Am. J. Vet. Res. 57:308-312.

Paape, M.J., Capuco, A.V., Guidry, A.J. and Burvenich, C. 1996. Morphology, function and adaptation of mammary cells in normal and disease states. J. Animal Sci. (In Press).

Cifrian, E., Guidry, A.J., Bramley, A.J., Norcross, N.L., Bastida-Coruera, F.D. and Marquardt, W.W. 1996. Effect of β-toxin *Staphylococcus aureus* cytotoxicity, proliferation and adherence to bovine mammary epithelial cells. Vet. Microbiol. (In Press).

Smits, E., Cifrian, E., Guidry, A.J., Rainard, Pl, Burvenich, C. and Paape, M.J. 1996. Cell culture for studying bovine neutrophil diapedesis. J. Dairy Sci. (In Press).

Cifrian, E., Guidry, A.J. and Marwuardt, W.W. 1996. Effect of antibodies to α and β toxins, cell wall and exopolysaccharide capsule on the cytotoxicity and adherence of *Staphylococcus aureus* to bovine mammary epithelial cells. Am. J. Vet. Res. (In Press).

Almeida, R.A., Matthews, K.R., Cifrian, E., Guidry, A.J. and Oliver, S.P. 1996. *Staphylococcus aureus* invasion of bovine mammary epithelial cells. J. Dairy Sci. (In Press).

O'Brien, C.N. and Guidry, A.J. 1996. Formulation of poly (D, L-lactide-co-glycolide) microspheres and their ingestion by bovine leukocytes. J. Dairy Sci. (In Press).

Guidry, A.J. and O'Brien, C.N. 1996. A method for measuring specific antibodies in bovine lacteal secretions during the dry period. J. Dairy Sci. (In Press).

Keys, J.E., Guidry, A.J. and Cifrian, E. 1996. The use of flow cytometry and fluorescein-labeled antibodies to measure specific milk proteins in bovine mammary epithelial cells. Cytometry. (In Press).

Keys, J.E., Cifrian, E., Guidry, A.J. and Farrell, H.M. 1996. Bovine mammary explants versus primary cell cultures: Effect of bovine somatotrophin and insulin-like growth factor-I on protein and DNA synthesis. In Vitro Cell. Dev. Biol. (In Press).

Dolores E. Hill
Research Parasitologist

CRIS 1265-32000-049, Strategies to Control Swine Parasites Affecting Food Safety

Progress: A thiol and a metalloprotease from *T. suis* were characterized and cloned. These enzymes may be involved in parasite feeding behavior or migration in the host intestine. They are targets of immunological and biochemical regulation. The adult worm excretory/secretory products, from which these enzymes are derived, are immunogenic and protect swine from infection. A feed efficiency study showed improved gain in immunized and challenge swine when compared to controls. There is also a reduced risk of secondary bacterial-induced disease of the colon that lowers the chance of carcass contamination with pathogenic bacteria.

Plans: Because of changing laboratory priorities, Dr. Hill will discontinue work on this CRIS project as of October 1996.

Outside Funding:

National Pork Producers Council Grant with J.F. Urban and R. Fetterer on protective antigens from culture fluids of the adult swine whipworm, *Trichuris suis*. 1995-1996

Cooperators:

R. Fetterer, ARS, USDA, Beltsville, MD
H. Ray Gamble, ARS, USDA, Beltsville, MD
M. Rhoads, ARS, USDA, Beltsville, MD
J. Sakanari, Univ. CA-SF, San Francisco, CA
R. Sawyer, BioPharm Ltd, Charleston, S.C.
A. Scott, Johns Hopkins School of Public Health and Hygiene, Baltimore, MD
L. Mansfield, Michigan State Univ., East Lansing, MI
J. Donnelly, Merck Sharp & Dohme Laboratory, Westpoint, PA
R. Pollack, Harvard School of Public Health and Hygiene, Boston, MA
T. Yoshino, Univ. Wisconsin, Madison, WI

Peer-Reviewed Publications (1993-present):

Hill, D.E., Gamble, H.R., Rhoads, M.L., Fetterer, R.H., Urban, J.F. 1993. *Trichuris suis*: A zinc metalloprotease from culture fluids of adult parasites. Experimental Parasitology, 77:170-178.

Fetterer, R.H. and Hill, D.E. 1993. The occurrence of phenol oxidase activity in female *Trichuris suis*. Journal of Parasitology. 79:155-159.

Fetterer, R.H. and Hill, D.E. 1994. Localization of phenol oxidase in female *Trichuris suis*. Journal of Parasitology. 80:952-959.

Hill, D.E., Fetterer, R.H., Romanowski, R.D. and Urban, J.F. 1994. The effect of immunization of pigs with *Ascaris suum* cuticle components on the development of resistance to parenteral migration during a challenge infection. Veterinary Immunology and Immunopathology. 42:161-169.

Hill, D.E. Donnelly, J.J., Lok, J.B., Khatami, M. and Rockey, J.H. 1996. A surface glycolipid masking cuticular antigens of *Onchocerca lienalis* microfilariae. Experimental Parasitology. (In Press).

Hill, D.E., Fetterer, R.H. and Urban, J.F. 1996. *Trichuris suis*: Further characterization of a culture fluid derived zinc metalloprotease from adult parasites. Experimental Parasitology. (In Press).

Hill, D.E. and Zarlenga, D.S. 1996. *Taenia crassiceps*: Immunological responses to a cloned antigen in the mouse host. Veterinary Parasitology. (In Press).

Hill, D.E. 1996. Analysis of cell populations in the peritoneal cavity of *Taenia crassiceps* infected mice. Parasite Immunology. (In Press).

Hill, D.E., Romanowski, R.D. and Urban, J.F. 1996. A *Trichuris*-specific diagnostic antigen from culture fluids of *Trichuris suis* adult worms.** Veterinary Parasitology. (In Press). **Patent application submitted.

Hill, D.E. 1996. Thiol protease activity from *Trichuris suis* adult worms. Molecular and Biochemical Parasitology. (Submitted).

Hill, D.E. 1996. Developmental regulation of a thiol protease from *Trichuris suis*. Molecular and Biochemical Parasitology. (Submitted).

Urban, J.F., Hill, D.E., Romanowski, R.D. and Fetterer, R.H. 1996. Development of immunity to *Trichuris suis* in swine. Parasite Immunology. (Submitted).

Mark C. Jenkins
Microbiologist

CRIS 1265-32000-050, Prevention & Therapy for Protozoan Parasites Affecting Food Animals, Food Safety, and Public Health

Coccidiosis (40%)

Progress: Unlike non-attenuated coccidia, *Eimeria* treated with an optimum dose of gamma irradiation do not cause clinical signs of coccidiosis in inoculated chickens, but do confer complete immunity to challenge infection in both one and seven day old chickens. Research with gamma-irradiated *Eimeria* parasites showed that the infected host cell and intracellular sporozoite metabolism was critical to development of protective immunity. Identified a species of cross-reactive *Eimeria* sporozoite antigen (EASZ240) and expressed it in yeast and *Salmonella*. Used yeast-derived EASZ240 to immunize chickens against *E. acervulina* challenge infection.

Plans: Will develop a "gel delivery" system for inoculating one-day-old chickens with irradiated *Eimeria* oocysts that may be used in place of non-attenuated coccidia (e.g. Coccivac, Immunocox). Study mRNA and antigens produced by metabolizing *Eimeria* sporozoites and clone cDNA encoding these components for testing in vaccination trials against coccidiosis. Expand vaccination trials using EASZ240 expressed in *Salmonella* and fowlpox virus (in collaboration with Nippon-Zeon, Inc. Japan) so that natural boosting may occur in the field.

Cryptosporidiosis (40%)

Progress: Identified and expressed two cDNA molecules encoding neutralizing 15 kDa antigens of *Cryptosporidium parvum* sporozoites. These cDNA were expressed in the yeast vector *Pichia pastoris* for eukaryotic expression of recombinant protein because *Escherichia coli*-derived recombinant antigen did not elicit "protective" immune serum for treating cryptosporidiosis. Utilized a gene-gun immunization technique for injecting plasmid DNA encoding a CP15/60 recombinant antigen in ruminant animals. Anti-CP15/60 responses were observed in serum and colostrum from gene-gun immunized animals. The serum and colostrum reacted with native CP15 antigen and bound the surface of *Cryptosporidium parvum* sporozoites. Developed a semi-quantitative PCR method for assessing infection levels in mice challenged with *Cryptosporidium parvum* oocysts. This technique will allow testing of hyperimmune serum and colostrum for passive immunity against relevant challenge doses of cryptosporidial parasites. Developed a PCR-based method for genus and species-specific identification of cryptosporidial organsims.

Plans: Will utilize gene-gun approach to immunize cows prior to drying off with recombinant CP15/60 plasmid DNA and test colostrum and serum for reactivity with CP15/60 antigen and sporozoites. This colostrum and serum will be tested for conferring passive immunity against cryptosporidiosis in neonatal mice. Will also test this gene injection method in preparturient mice to test for natural passive immunity against cryptosporidiosis in the neonatal mouse model. Will immunize cows with yeast-derived CP15/60 and CP15 recombinant antigen for production of neutralizing immunoglobulin. This colostrum and serum will be tested in the neonatal mouse challenge model. Will further refine the PCR-based detection and speciation method for identifying *Cryptosporidium* oocysts in environmental samples to ascertain viability and sources of the parasite.

CRIS 1265-32000-045, Epidemiology & Control of Toxoplasma, Trichinella, & Related Parasites in Domestic Animals

Neosporosis (20%)

Progress: Developed a PCR-based detection method for detecting *Neospora* parasites in brains of infected mice. This technique is a significant advance over previous technologies (e.g. immunohistochemical staining, indirect immunofluorescence) because it is more sensitive and specific. Cloned the first recombinant antigens from *Neospora* and developed a cost effective assay for diagnosing acute neosporosis in cattle using antigens expressed in *Escherichia coli*, thus obviating culturing of *Neospora* organisms for preparing diagnostic antigen. This assay is also more specific because the recombinant antigens, unlike more complex native antigen preparations, do not cross-react with constituents from closely related protozoa, particularly *Toxoplasma gondii*.

Plans: Will use recombinant *Neospora* antigens to follow neosporosis over several calving cycles in a dairy herd that is experiencing neosporosis-associated abortion. These studies will help elucidate the relative importance of congenital transmission of *Neospora* and the rate of multiple abortions in dairy cattle and may help veterinarians in making recommendations concerning infected cows. Will use PCR-based assay to (a) confirm neosporosis-associated abortion in dairy cows and (b) develop a mouse model for congenital transmission of *Neospora caninum*. The former may provide confirmatory diagnosis of abortion in dairy cattle most cases which remain undiagnosed. The latter will allow for testing of recombinant and native *Neospora* antigens as vaccines against neosporosis in dairy cattle.

Outside Funding:

CRADA with K. Kamogawa, Nippon-Zeon, Inc., Tokyo, Japan. on development of coccidiosis vaccine using recombinant fowlpox virus. 1994-1997.
Trust Fund Cooperative Agreement with R. Gill, British-Technology Group, Inc., Gulph Mills, PA, on production of recombinant *Eimeria* antigens for vaccination against coccidiosis. 1995-1996.
Trust Fund Cooperative Agreement with C. Ricks, EMBREX, Inc., Morristown, NC, on in ovo vaccination against coccidiosis. 1995-1996.
Cooperative Agreement with H. Hughes, Mallinckrodt Animal Health, Inc., Mundelein, MN and Biregional Research Development Consortium on development of mouse model for neosporosis. 1996-1997.
USDA, NRI Grant with H. Lillehoj on cloning of cytotoxic T cells mediating protection to coccidia. 1992-1995.
BARD Grant with H. Lillehoj on molecular and cellular characterization of protective *Eimeria acervulina* merozoite antigens. 1993-1995.
USDA, NRI Grant with H. Lillehoj on molecular cloning of *Eimeria* sporozoite receptor proteins involved in host lymphocytes. 1995-1997.

Cooperators:

M. Abrahamsen, Univ. of Minnesota, Minneapolis, MN.
J. McGuire, ENZON, Inc., Piscataway, NJ.
E. McGruder, Lilly, Greenfield, IN.
H. Profous-Chulcheka, Merck, Inc., Rahway, NJ.
S. Johnston, Univ. of Texas Southwestern Medical School, Dallas, TX
H. Hughes, Mallinckrdot Animal Health, Mundelein, MN.
D. Colley, Centers for Disease Control, Atlanta, GA.
E.H. Lee, Vetech, Inc., Guelph, Ontario, Canada
M. Dekich, Perdue Farms, Inc. Salisbury, MD.
J. Barta, Univ. of Guelph, Guelph, Ontario, Canada
H. Danforth, Parasite Biology and Epidemiology Laboratory, LPSI, ARS, Beltsville, MD
P. Allen, Parasite Biology and Epidemiology Laboratory, LPSI, ARS, Beltsville, MD
P. Augustine, Parasite Biology and Epidemiology Laboratory, LPSI, ARS, Beltsville, MD
C. Chappell, Univ. of Texas School of Public Health, Dallas, TX
D. Lowery, Upjohn, Inc., Kalamazoo, MI
R. Menon, Galagen, Inc., Minneapolis, MN
R. Wall, Gene Evaluation and Mapping Laboratory, LPSI, ARS, Beltsville, MD
A. Guidry, Immunology and Disease Resistance Laboratory, LPSI, ARS, Beltsville, MD

Peer-Reviewed Publications (1993-present):

Jenkins, M.C., Seferian, P.G., Augustine, P.C. and Danforth, H.D. 1993. Protective immunity against coccidiosis elicited by radiation-attenuated *Eimeria maxima* sporozoites that are incapable of asexual development. Avian Diseases 37:74-82.

Augustine, P.C., Danforth, H.D. and Jenkins, M.C. 1993. Avian *Eimeria*: Effects of gamma irradiation on development of cross-species immunity in foreign and natural host birds. Avian Diseases 37:349-357.

Jenkins, M.C., Fayer, R. Tilley, M. and Upton, S. 1993. Cloning and expression of a cDNA encoding epitopes shared by 15- and 60-kilodalton proteins of *Cryptosporidium parvum* sporozoites. Infection and Immunity. 61:2377-2382.

Jenkins, M.C., Augustine, P.C., Danforth, H.D., Seferian, P.G. and Barta, J.R. 1993. *Eimeria* oocysts exposed to gamma irradiation induce protective immunity in the absence of merogony: Relevance to Vaccine Development. Proceedings of the VIth International Coccidiosis Conference. Guelph, Ontaria, Canada. p. 109-117.

Danforth, H.D., Augustine, P.C. and Jenkins, M.C. 1993. A review of progress in coccidial vaccine development. Proceedings of the VIth International Coccidiosis Conference. Guelph, Ontaria, Canada. p. 49-60.

Augustine, P.C., Danforth, H.D., Jenkins, M.C. and Barta, J.R. 1993. Avian *Eimeria*: Ability of sporozoites to elicit cross species protection in foreign host birds. Proceedings of the VIth International Coccidiosis Conference. Guelph, Ontaria, Canada. p. 105-108.

Augustine, P.C., Danforth, H.D. and Jenkins, M.C. 1993. Cross-species protection elicited in chickens by the turkey coccidium *Eimeria adenoides* is destroyed by gamma irradiation. Poultry Science. 72: 21.

Bjerkas, I., Jenkins, M.C. and Dubey, J.P. 1994. Analysis of *Neospora caninum* antigens by Western immunoblotting and immunogold electron microscopy. Clinical and Diagnostic Laboratory Immunology. 1:214-221.

Jenkins, M.C. and Fayer, R. 1994. DNA sequence encoding surface protein of *Cryptosporidium parvum*. U.S. Patent Office Application 08/068,396.

Jenkins, M.C. and Fayer, R. 1994. Cloning and expression of cDNA encoding a 15 kDa *Cryptosporidium parvum* sporozoite protein. Molecular and Biochemical Parasitology. 71:149-152.

Jenkins, M.C., Chute, B.C., Danforth, H.D. and Lillehoj, H.S. 1994. Gamma-irradiated and non-irradiated *Eimeria tenella* sporozoites exhibit differential uracil uptake and expression of a 7-10 kDa metabolic antigen. Experimental Parasitology. 80:645-653.

Danforth, H.D., Augustine, P.C., Barta, J.R. and Jenkins, M.C. 1994. In vitro and in vivo immunolabeling of sporozoites, schizonts, and sexual stages of *Eimeria acervulina* and *E. tenella* by a species and stage cross-reactive monoclonal antibody. Parasitology Research. 80:588-593.

Danforth, H.D., Augustine, P.C. and Jenkins, M.C. 1994. Vaccine Development: Current Position and Perspectives. Proceedings of Siposio Internacional sobre Coccidiose. p. 125-140.

Jenkins, M., Kerr, D., Fayer, R. and Wall, R. 1995. Serum and colostrum antibody responses induced by jet-injection of sheep with DNA encoding a *Cryptosporidium parvum* antigen. Vaccine. 13:1658-1664.

Lally, N., Jenkins, M.C. and Dubey, J.P. 1996. Development of a polymerase chain reaction assay for diagnosis of neosporosis using the *Neospora caninum* 14-3-3 gene. Molecular and Biochemical Parasitology. (In Press).

Lally, N., Jenkins, M.C. and Dubey, J.P. 1996. Evaluation of two *Neospora caninum* recombinant antigens for use in an ELISA for the diagnosis of bovine neosporosis. Clinical and Diagnostic Immunology Laboratory. (In Press).

Kaspers, B., Lillehoj, H., Jenkins, M.C. and Pharr, G.T. 1996. Chicken interferon-mediated induction of major histocompatibility complex Class II antigens on peripheral blood monocytes. Veterinary Immunology and Immunopathology. (In Press).

Augustine, P.C. and Jenkins, M.C. 1996. Effect of conditioned media from several cell types on invasion by *Eimeria adenoeides* sporozoites. Journal of Eukaryotic Microbiology. (In Press).

H. Lillehoj
Microbiologist

CRIS 1265-32000-050, Prevention and Therapy for Protozoan Parasites Affecting Food Animals, Food Safety and Public Health (70%)

Progress: Provided basic information on the interaction of effector cells and lymphokines involved in host defense at intestinal surfaces and investigated the role of natural killer and intraepithelial lymphocytes in coccidiosis. Developed a protocol to purify chicken IL-2 and interferon and conducted biochemical and functional characterizations. Demonstrated that lymphokines, such as IL-2 and IFN, enhance anti-coccidial immunity. Developed various monoclonal antibodies which detect intestinal lymphocyte subpopulations and used these antibodies to define various immunity factors which regulate local immune responses to coccidiosis and other microbial pathogens.

Plans: Characterize in detail the nature of host-parasite interactions leading to protective immunity, identify effector cells and lymphokines mediating protection, Identify the nature of sporozoite receptors involved in lymphocyte invasion and develop recombinant vaccine which will block early interaction of sporozoites and host, characterize immunomodulation induced by *in ovo* treatment of cytokines, characterize protective host immunity to murine coccidiosis using knockout mice model.

CRIS 1265-31320-012, Identification and Mapping of Genes Involved in Parasitic Disease Resistance/Suceptibility (30%)

Progress: Demonstrated importance of MHC and non-MHC gene control of coccidiosis susceptibility using MHC-congenic chickens. Demonstrated a relationship between host genetics and the efficacy of recombinant antigen immunization. Demonstrated certain aspects of genetic control of host immune response to coccidian parasites. These included tumor-necrosis factor and gamma-interferon production in genetically defined chickens which show different levels of coccidia disease susceptibility.

Plans: Investigate coccidiosis susceptibility in commercial broiler chickens and identify microsatellite markers which correlate with disease resistance. Investigate the role of major histocompatibility complex and non-MHC genes in controlling disease susceptibility. Identify immune responses including cell-mediated responses and cytokine/lymphokine production in susceptible and resistance chicken lines.

Outside Funding::

USDA, NRI Grant with M. Jenkins on cloning of cytotoxic T cells mediating protection to Coccidia. 1992-1995.
BARD Grant with M. Jenkins on molecular and cellular characterization of protective *Eimeria acervulina* merozoite antigens. 1993-1995.
USDA, NRI Grant with M. Jenkins molecular Cloning of *Eimeria* Sporozoite receptor protein involved in host lymphocytes. 1995-1997.
Egyptian Education Bureau grant for research of K. Zyan. 1994-1996.
CRADA with C. Ricks, EMBREX, Inc., Morristown, NJ, on immune enhancement induced by in ovo injection of chicken cytokines. 1995-1998.

Cooperators:

M. Jenkins, Immunology & Disease Resistance Laboratory, LPSI, ARS, Beltsville, MD
P. Allen, Parasitology and Epidemiology Laboratory, LPSI, ARS, Beltsville, MD.
D. Zarlenga, Immunology & Disease Resistance Laboratory, LPSI, ARS, Beltsville, MD.
J. Urban, Jr., Immunology & Disease Resistance Laboratory, LPSI, ARS, Beltsville, MD.
L. Bacon & H. Cheung. ADOL, USDA-ARS, East Lansing, MI.
R. Meinersmann, PMSRU, ARS, USDA, Athens, GA.
J. Marsh, Dept of Microbiology, Cornell Univ., New York.
L. Keller, Poultry Diagnostic Lab, Univ. PA, Kennett Square, PA.
S. Lamont: Dept of Animal Science, Iowa State Univ., Ames, IA.
P. Staeheli, Dept of Virology, Univ. of Freiburg, Freiburg, Germany.
B. Kaspers, Institute of Physiology, Univ. of Munich, Munich, Germany
Imre Olah, Dept of Anatomy, Semmelweis Univ. Medical School, Budapest, Hungary.
J. Y. Han, Coll. Agricultural Biotechnology, Seoul National Univ., Suwon, Korea.
K. Sasai, Dept of Internal Medicine, Univ. of Osaka Prefecture, Osaka, Japan.
A. Hemphill, Inst. Parasitology & Vet. Med., Univ. of Berne, Berne, Switzerland.
J. Sanchez-Garcia & W. McCormack, Dept. Pathol. & Lab. Med., Univ. FL Coll. Med., FL.
Marlene Emara, College of Agricultural Sciences, Univ. of Delaware, Newark, Del.
W. E. Briles, Dept of Biological Sciences, Northern Illinois Univ., Dekalb, IL.
Sonia Jakelow, Biomarkers & Prevention Branch, NCI, NIH. Rockville, MD.
D. Poulik, Purdue Farms, Inc. Salisbury, MD.
P. Johnston. EMBREX Inc., Res. Tri Park, NC.

Peer-reviewed Publications (1993-present):

Lillehoj, H.S. 1993. Avian Gut-Associated Immune system. Implication in coccidial vaccine development. Poul. Sci. 72:1306-1311.

Lillehoj, H.S. 1993. Immune responses to coccidian parasites. Proc. VIth International Coccidia Conference. J. R. Barta and Fernando, M. A. ed., University of Guelph, Canada, p. 11-18.

Lillehoj H.S. and Trout, J.M. 1993. Coccidia. A review of recent advances on immunity and vaccine development. Avian Pathology. 22:3-21.

Isobe, T. and Lillehoj, H.S. 1993. Effect of immunosuppression on chicken coccidiosis. Proc. of the First Asian Conference on Avian Coccidiosis, A. Arakawa ed., University of Osaka, Osaka, Japan. p. 127-136.

Kaspers, B, Lillehoj, H.S. and Lillehoj, E.P. 1993. Chicken macrophages and thrombocytes share a common cell surface antigen defined by a new monoclonal antibody. Vet. Immunol. Immunopathol. 36: 333-346.

Lillehoj, H.S., Isobe, T. and Weinstock, D. 1993. Tissue distribution and cross-species reactivity of new monoclonal antibodies detecting chicken T lymphocytes and macrophages. Avian Immunology in Progress. F. Coudert, ed. INRA, Paris, France, p. 37-42.

Lillehoj, H.S. 1993. Avian interleukin-2 and interferon. Avian Immunology in Progress. F. Coudert, ed. INRA, Paris, France, p. 105-112.

Lillehoj, H.S. and Nichols, M. 1993. Contrasting effects of corticosterone on anti-eimerial host immunity in two genetically disparate chickens. Avian Immunology in Progress. F. Coudert, ed. INRA, Paris, France, p. 251-256.

Lillehoj, H.S., Kaspers, B. and Myers, T.J. 1993. Biochemical and functional characterizations of avian gamma-interferon and interleukin-2. Avian Immunology in Progress. F. Coudert, ed. INRA, Paris, France, p. 131-136.

Isobe, T. and Lillehoj, H.S. 1993. Dexamethasone suppresses T-cell mediated immunity and enhances disease susceptibility to *Eimeria mivati* infection. Vet Immunol. Immunopathol. 39:431-446.

Lillehoj, H.S., Lindblad, E.B. and Nichols, M. 1993. Adjuvanticity of Dimethyl Diooctadecyl Ammonium Bromide, Complete Freund's Adjuvant and Corynebacterium parvum with respect to host immune response to coccidial antigens. Avian Dis. 37:731-740.

Martin, H.S., Lillehoj, H.S., Kaspers, B. and Bacon, L.D. 1993. Antigen-specific proliferation and interferon production induced by coccidia infection. Poul Sci. 72:2084-2094.

Lillehoj, H.S. 1994. Analysis of *Eimeria acervulina*-induced changes in intestinal T lymphocyte subpopulations in two inbred chickens showing different levels of disease susceptibility to coccidia. Research Vet. Science. 56:1-7.

Lillehoj, H.S., Sasai, K.S. and Matsuda, H. 1994. Development and characterization of chicken-chicken B-cell hybridomas secreting monoclonal antibodies detecting sporozoite and merozoite antigens of *Eimeria*. Poul Sci. 73:1685-1693.

Martin, A., Lillehoj, H.S., Kaspers, B. and Bacon, L.D. 1994. Mitogen-induced lymphocyte proliferation and interferon production induced by coccidia infection. Avian Dis. 38:262-268.

Lillehoj, H.S. and Trout, J.M. 1994. CD8+ T Lymphocyte-Coccidia Interactions: Role in transport and Cytotoxic Function in Avian Coccidiosis. Parasitology Today. 10:10-14.

Lillehoj, H.S. 1993. Can Susceptibility to Coccidiosis Decreased by Increasing Immunocompetence?. World Poultry Journal. Special Issue on Coccidiosis, August. p. 28-30.

Lillehoj, H.S. and Martin, A. 1994. Flow cytometry and fluorescence-activated cell sorting. Antibody Techniques, V. Malik and E.P. Lillehoj eds., p. 291-305, Academic Press.

Trout, J.M. and Lillehoj, H.S. 1994. *Eimeria acervulina*: Evidence for the involvement of $CD8^+$ T lymphocytes in sporozoite transport and host protection. J. Parasitol. 44:71-84.

Kaspers, H.S., Lillehoj, H.S., Jenkins, M.C. and Pharr, G.T. 1994. Chicken interferon-mediated induction of major histocompatibility complex class II antigens on peripheral blood monocytes. Vet. Immuno. Immunopathol. 44:71-84.

Sasai, K., Lillehoj, H.S., Wergin, W.P., Matsuda, H., Miyamoto, T., Fukata, T., Baba, E. and Arakawa, A. 1994. Chicken monoclonal antibody to sporozoite of *Eimeria acervulina* recognized the conoid antigen by immuno-electro microscopy. The 2nd International Coccidiosis Conf., p. 11-16.

Lillehoj, H.S. 1995. Monoclonal antibodies against chicken T-lymphocytes, US 005449610, Approved Sep. 12, 1995.

Jenkins, M.C., Chute, B., Danforth, H. and Lillehoj, H.S. 1995. Gamma-irradiated and non-irradiated *Eimeria tenella* sporozoites exhibit differential uracil uptake and expression of a 7-10 kDa metabolic antigen. Exp. Parasitol. 80:645-653.

Martin, A. , Awadella, S. and Lillehoj, H. S. 1995. Characterization of cell mediated responses to *Eimeria acervulina* sporozoite and merozoite antigens. Avian Dis. 39:538-547.

Trout, J.M. and Lillehoj, H.S. 1995. *Eimeria acervulina* infection: Evidence for the involvement of CD8+ T Lymphocytes in sporozoite transport. Poul. Sci. 74:1117-1125.

Lillehoj, H.S. and Nichols, M. 1995. Chicken monoclonal antibodies blocking coccidia invasion of host lymphocytes. Patent filing. Docket No. 0253.93. Chicken monoclonal antibodies specific for coccidial antigens involved in invasion of host lymphocytes".

Lillehoj, H.S. 1995. New approaches toward coccidial development. Proc. the 46th North Central Avian Disease Conference and Symposium on New Vaccines and Delivery System. Minneapolis, Minn. p. 20-24.

Zhang, S. Lillehoj, H.S. and Ruff, M.D. 1995. In vivo role of tumor necrosis factor in *Eimeria tenella* infection. Avian Diseases. 39:859-866.

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Lillehoj, H.S. 1995. The poultry scientists and agromedicine:Looking into the third millennium. J. Agromed. 2:14-18.

Sasai, K., Lillehoj, H.S., Matsuda, H., Hanioka, Y., Fukata, T., Baba, E. and Arakawa, A. 1995. Identification of a common conoidal determinant among different *Eimeria* species with a chicken monoclonal antibody to *Eimeria acervulina*. Proc. 15th Int. Conf. World Asso. Vet. Parasitology. Page C14.

Sasai, K., Lillehoj, H. Matsuda, H., Wergin, W. P. 1996. Characterization of a chicken monoclonal antibody that recognizes the apical complex of *Eimeria acervulina* sporozoites and partially inhibits sporozoite invasion of CD8+ T lymphocytes by sporozoites in vitro. J. Parasitol. 82:82-87.

Trout, J.M. and Lillehoj, H.S. 1996. T Lymphocyte roles during *Eimeria acervulina* and *Eimeria tenella* infections. Vet. Immunol. Immunopathol. (In Press).

Lillehoj H.S. and Trout, J.M. 1996. Avian gut-associated lymphoid tissues and intestinal immune responses to *Eimeria* parasites. Clin Microbiolol Review. (In Press).

Joan K. Lunney
Supervisory Immunogeneticist

CRIS 1265-31320-012, Identification and Mapping of Genes Involved in Parasitic Disease Resistance/Suceptibility (60%)

Progress: Analyses for genes which encode resistance to *Toxoplasma gondii* have recently been initiated using the NIH minipig model. Initial dose response studies have established a reproducible oocyst dose for primary infections. The first full test of genetic resistance has potentially indicated that *Toxoplasma gondii* resistant pigs can be identified and that the genes encoding resistance do not map to the swine leukocyte antigen complex. Developed procedures to compare genomic DNA from pigs with different performance traits using genetically directed representational difference analysis (GRADA) .

Plans: Tests of genetic resistance to *Toxoplasma gondii* are continuing. Phenotypic studies are underway to determine whether T cell associated cytokines, particularly interferon-gamma (IFNγ) and interleukin-4 (IL-4), IL-10 and IL-12, regulate resistance to this parasitic infection. Samples for cytokine bioassays and mRNA analyses have been collected during the earlier infection studies so that quantitation of these responses can be performed. These analyses should not only lead to genetically resistant pigs but should also help to define protective immune responses against this parasitic infection and thus help to design new vaccines and biopharmaceuticals. Will use linkage mapping and GRADA techniques to compare genomic DNA of parasite resistant versus susceptible individuals to identify genes which confer disease resistance.

CRIS 1265-32000-049, Strategies to Control Swine Parasites Affecting Food Safety (40%)

Progress: Developed panels of immune reagents to assess cellular immune responses of swine during parasitic infection. Standardized panels of monoclonal antibodies that react with lymphoid cell subsets through collaborations established for the First and Second International Swine CD Workshops. Prepared competitive molecular probes to quantitate mRNA levels of swine IL-2, IL-10, and IL-12 and IFNγ and used probes to assess cytokine mRNA levels in tissues from parasite infected pigs. Used cloned and expressed swine cytokines to prepare cytokine specific monoclonal antibodies for use in ELISA and ELISPOT assays. During *Toxoplasma gondii* infections archived local tissue samples and stimulated cell supernatants for analyses of cytokine control of protective immune responses.

Plans: Finalize preparation of cytokine probes for interferon-gamma, IL-4, IL-15, tumor necrosis factor-alpha, and other cytokines as required by current immune studies. Prepare monoclonal antibodies for IL-4 and, if necessary, additional monoclonal antibodies for IL-10 (2 antibodies have already been prepared) and develop sensitive

ELISA assays for activity analyses. Use these reagents in parasitic disease sudies to identify regulatory cytokines that stimulate protective anti-disease responses and that determine how genetically resistant pigs are able to resist foodborne parasitic infections.

Outside Funding:

USDA NRI grant with C. Louis, Univ. Minnesota for Production and use of a swine chromosome 6 genomic library. 1993-1995.
USDA NRI grant renewal with C. Louis, Univ. Minnesota for Development of reagents for identifying QTL on swine chromosome 6. 1995-1997.
National Pork Producers Council competitive grant with L. S. Mansfield and J. F. Urban, Jr. for Production of a new tool to assess effective swine disease responses. 1995-1996.
CRADA with Upjohn Animal Health with S. Martin for Production and use of porcine cytokines for animal disease studies. 1994-1999.

Cooperators:

A. Saalmueller, Fed. Res. Center for Viral Diseases of Animals, Tuebingen, Germany
M. Pescovitz, Dept. Surgery, Immunol. and Microbiol., Indiana Univ., Indianapolis, IN
F. Zuckermann, Dept. Vet. Pathobiology, Univ. IL-Urbana, Urbana, IL
D. Strom, CSIRO, Melbourne, Australia
S. Martin, Pharmacia/Upjohn Animal Health, Kalamazoo, MI
L. Mansfield, Dept. Large Animal Medicine, Michigan State Univ., East Lansing, MI
M. Murtaugh, Dept. Vet. PathoBiology, Univ. Minnesota, Minneapolis, MN
J.P. Dubey, Parasite Biology and Epidemiology Laboratory, LPSI, Beltsville, MD
M. Myers, FDA Center for Vet. Research, Beltsville, MD
F. Abel Ponce deLeon, Univ. Massachusetts, Amherst, MA
C. Louis, L. Schook, Dept. Vet. PathoBiology, Univ. Minnesota, Minneapolis, MN

Peer Reviewed Publications (1993-present)

Lunney, J.K. 1993. Characterization of swine leukocyte differentiation antigens. Immunology Today. 14:147-148.

Madden, K.B., Moeller, R.F., Jr., Goldman, T. and Lunney, J.K. 1993. *Trichinella spiralis*: Genetic basis and kinetics of the anti-encysted muscle larval response in miniature swine. Exp. Parasitol. 77:23-35.

VanCott, J.L, Brim, T.A., Lunney, J.K. and Saif, L.J. 1993. Contribution of antibody-secreting cells induced in mucosal lymphoid tissues of pigs inoculated with respiratory or enteric strains of coronavirus to immunity against enteric coronavirus challenge. J. Immunol. 152:3980-3990.

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Lunney, J.K. 1994. Editor: Special Issue: Porcine Immunology. Vet. Immunol. Immunopathol. 43:1-333.

Lunney, J.K. 1994. The Swine Leukocyte Antigen (SLA) Complex. Vet. Immunol. Immunopathol. 43:19-28.

Baker, D., Bryant, J.D., Urban, J.F. and Lunney, J.K. 1994. Swine immunity to selected parasites. Vet. Immunol. Immunopathol. 43:127-134.

Lunney, J.K., Walker, K., Goldman, T., Aasted, B., Bianchi, A., Binns, R.M., License, S., Bischof, R., Brandon, M., Blecha, F. Kielian, T.L., McVey, D.S., Chu, R.M., Carr, M.M., Howard, C.J., Sopp, P., Davis, W.C., Dominguez, J., Canals, A., Sanchez Vizcaino, J.M., Kim, Y.B., Laude, H., Mackay, C.R., Magnusson, U., McCullough, K., Misfeldt, M., Murtaugh, M., Molitor, T., Choi, C., Pabst, R., Parkhouse, R.M., Denham, S., Yang, H., Pescovitz, M., Pospisil, R.,Tlaskalova, H., Saalmueller, A., Salmon, H., Sachs, D., Arn, S., Shimizu, M., Stokes, C., Stevens, K., Valpotic, I., Zuckermann, F.A. and Husmann, R. 1994. Overview of the first international workshop to define swine leukocyte cluster of differentiation (CD) antigens. Vet. Immunol. Immunopathol. 43:193-206.

Lunney, J.K., Walker, K. and Goldman, T. 1994. Analyses of anti-human CD monoclonal antibodies or cross reactions with swine cell antigens. Vet. Immunol. Immunopathol. 43:207-210.

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Max Paape
Dairy Scientist

CRIS 1265-32000-048, Reduce Mastitis Incidence and Antibiotic Use by Enhancing Cow's Natural Defenses

Progress: Ionic calcium was shown to play an important role in the process of phagocytosis by bovine neutrophils in the presence of opsonins, and nonopsonic phagocytosis might be an event that is less dependent on calcium. Intracellular calcium influx and protein tyrosine phosphorylation was induced by IgG_2 and IgM binding to bovine neutrophils. Binding of these opsonic antibodies may trigger the pathway for effective neutrophil phagocytosis and prevention of mastitis. Reagents were developed to quantitate neutrophil cell surface receptors involved in adherence, phagocytosis and oxidative burst. Unopsonized zymosan acted in a divalent cation-dependent manner to identify the CR3 receptor. Isolated and purified bovine complement C3b to characterize CR1 receptors on bovine neutrophils. Showed that interferon-τ upregulated Fc receptors for IgG by de novo RNA transcription and protein synthesis. Migration of neutrophils caused shedding of nonspecific IgG_2 and IgM from their receptors and increased receptor expression, allowing for a more specific recognition between neutrophils and bacteria. Elucidated neutrophil surface molecules recognized by a panel of anti-bovine neutrophil monoclonal antibodies. Discovered presence of CD14 receptors on neutrophils in milk which may play a role in localized activation of mammary neutrophils through release of tumor necrosis factor. Results established important role of neutrophil receptors in mastitis and in development of vaccination strategies and production of immunotherapeutic reagents.

Plans: Cell surface antigens recognized by anti-bovine neutrophil monoclonal antibodies (MAB) will be further characterized by immunoprecipitation and competitive binding with human MAB that crossreact with bovine neutrophil cell surface antigens. The role of purified complement component C5a and bovine recombinant tumor necrosis factor on neutrophil phagocytosis, oxidative burst and Fc receptor expression will be determined. Using an ELISA developed for the chemoattractant C5a, the concentration of C5a in serum and milk will be determined following challenge with live *Escherichia coli*. The effect of inflammation of the mammary gland on nitration of tyrosine residues on proteins in milk and in neutrophils and free nitrotyrosine in milk will be assessed and their effect on in vitro mammary epithelial cell damage and neutrophil function will be determined. Using anti-bovine MAB to activation molecules on the neutrophil cell surface and MAB to mastitis pathogens, bispecific antibodies will be constructed for targeted lysis of mastitis pathogens by neutrophils. The bovine complement receptor-1 (CR1) will be cloned and a panel of monoclonal antibodies against the purified protein will be used for neutrophil intracellular signaling studies. Additionally, the molecular weight of bovine CR1 will be determined using western blotting techniques.

Outside Funding:

Food and Drug Administration Interagency Agreement with N. Alderson, CVM, on evaluation of antibiotic residue screening tests for use in milk from individual cows and goats. 1993-1997.

Cooperative Research with D. Westhoff, Dept. of Anim. Sciences, Univ. of Maryland, College Park for graduate studies of Y. Wang, S. Komaragiri, T. Nelson. 1979-present.

Cooperative Research with M. A. Brown, USDA-ARS, Booneville, AR on genetic evaluation of milk yield in beef cows. 1993-present.

Cooperative Research with I. Ades, Dept Zoology, Univ. of Maryland, College Park for graduate studies of A. Di Carlo. 1992-present.

Cooperative Study with Sporicidin Internationaland Univ. of Maryland with R. Peterson evaluation of product for teat dip. 1995-present.

Pending agreements with Y. Nakamura, Ajinomoto Co. Inc., Tokyo, Japan, on development of bi-specific antibodies for targeted lysis of mastitis pathogens by bovine neutrophils and on screening of newly developed pharmacological compounds for their effects on neutrophil function.

Cooperators:

R. Peters, Univ. of Maryland, College Park, MD
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P. Rainard and B. Poutrel, Insitut National de la Recherche Agronomique, Nouzilly, France
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L. Leino and E.-M. Lilius, Univ. of Turku, Turku, Finland
S. Soback and G. Ziv, Kimron Vet. Inst., Beit-Dagan, Israel
Cambridge Biotech, Worcester, MA. 1994-present
A. Capuco, GEML, ARS, USDA, Beltsville, MD
N. Talbot, GEML, ARS, USDA, Beltsivlle, MD
M. Worku, FDA, Bethesda, MD
M. Kehrli, Jr., NADC, Ames, IA
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Joseph Urban
Supervisory Microbiologist

CRIS 1265-32000-049, Strategies to Control Swine Parasites Affecting Food Safety

Progress: Parasite antigen specific antibody secreting cells (S-ACS) were detected in pig intestinal mucosa by ELISPOT. The assay demonstrated a distinct compartmentalization of the immune response to *Trichuris suis*; S-ACS were detected earlier and more prominently in the colonic lymph nodes and ileocecal Peyer's patches than in the mesenteric lymph nodes and distal Peyer's patches. Mucosal immunity was stimulated via nasal and colonic sensitization; ELISPOT was used to validate the efficiency of this route of intestinal sensitization. Immunization as an immunoprophylactic and growth enhancing procedure was demonstrated in parasite exposed pigs through feed efficiency studies.

Plans: Evaluate pig colonic lymphoglandular complexes as antigen processing structures; test protective immunity following direct mucosal sensitization; demonstrate effectiveness of immunity in an integrated control strategy; develop *in situ* assay of parsite metabolic changes in cytokine treated hosts; determine the cytokine response pattern in gut associated lymphoid tissue of protozoan infected (*Toxoplasma gondii* and *Cryptosporidium parvum*) versus helminth infected (*T. suis* and *Trichinella spiralis*) pigs.

CRIS 1265-32000-050, Prevention and Therapy for Protozoan Parasites Affecting Food Animals, Food Safety and Public Health

Progress: Demonstrated that exogenous IL-12 regulated resistance to *Cryptosporidium parvum* through an IFN-γ-dependent and immune system-independent mechanism, and that endogenous IL-12 controlled the severity of disease. A IFN-γ requirement for resistance to *C. parvum* was shown using specific neutralizing mAb and IFN-γ-Knock out (KO) mice lacking IFN-γ gene expression.

Plans: Describe the mechanism of resistance in mice deficient for particular cellular and molecular effector mechanisms; evaluate resistance/susceptibility in mice lacking perforin, IFN-γ, CD3e, B-cells, and nitric oxide using KO mice. Determine whether IL-12 induces developmental resistance in naive mice prior to parasite exposure or can act as an adjuvant during vaccination of dams to transfer passive immunity to pups.

Outside Funding:

National Pork Producers Counctil grants with D. Hill and R. Fetterer on protective antigens from culture fluids of the adult swine whipworm, *Trichuris suis*. 1995-1996.
National Pork Producers Council competitive grant with L. S. Mansfield and J. K. Lunney, for production of a new tool to assess effective swine disease responses. 1995-1996.
American Society of Microbiology grant with L. Mansfield for student travel and instructional grant.
National Institutes of Health grants with S. Rinehart, F. Finkelman and W. Gause for salary support for Visiting Scientist/Technician. 1994-present.
VA/MD Regional Veterinary College collaboritive research with M. Kellman and A. Zajac for Graduate student support stipends. 1993-1996.

Cooperators:

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P. Nansen, Royal Agricultural University, Copenhagen, Denmark

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D. Zarlenga
Microbiologist

CRIS 1265-32000-044, Immunobiology of Ostertagia ostertagi and other gastrointestinal nematodes of Cattle (35%)

Progress: Identified repetitive DNA sequences specific for each of the major parasite genera infecting cattle that can be used for non-invasive differentiation of pathogenic infections. Also developed probes for differentiating species within the genus *Haemonchus*. Developed a PCR based method to rapidly diagnose and quantify the pathogenic species of cattle nematodes using DNA from fecal eggs. Cloned and expressed bovine cytokines IL-12 and IL-15. IL-12 was previously shown to play an important role in controlling certain parasite infections in mouse models. Developed a genetically engineered antigen for use in the serodiagnosis of bovine and swine cysticercosis. A patent for this gene and gene products is pending. Developed method for generating competitor molecules for use in studying cytokine gene expression in bovine and used this method to synthesize competitor molecules for T-cell related cytokines IL-2, IL-4, IL-10, IL-12, IL-15, IFN-γ and the internal control molecules, HPRT and β-actin.

Plans: Use the competitor molecules as well as truncated forms of the parent molecules in RT-PCR and RNase protection assays to dissect changes in the bovine immune response resulting from parasite invasion. Study differences among animal groups characterized as susceptible and resistant to parasite infection. Generate sufficient quantities of bovine IL-12 and IL-15 to initiate studies on the use of these cytokines in prophylactic control of parasites and as adjuvants for administering recombinant-derived parasite-protective antigens.

CRIS 1265-32000-049, Strategies to control swine parasites affecting food safety (35%)

Progress: Developed PCR based method to differentiate *Trichinella* species used for epidemiological studies in Indiana, Illinois and by Agriculture Canada. It has also been used to determine the source of infection originating from cougar jerkey and in publishing the first reported natural infection in horses. Demonstrated the potential for multiple, non-synchronic infections of heterologous species within the same host suggesting the potential for hybridization among the domestic species and the freeze resistant species of this genus. Initiated studies to identify cryptic parasite gut antigens as targets for drug intervention or vaccine trials. Developed methodology and reagents for use in studying changes in cytokine expression in swine resulting from parasite invasion.

Plans: Determine the potential to introduce the freeze resistant phenotype of *Trichinella nativa* into the domestic cycle. Continue to develop noval targets for attenuating parasite infection by concentrating on cryptic gut associated proteins. Begin to study changes in gene transcription of swine cytokines that correlate with parasite infection utilizing previously developed reagents.

Outside Funding::

USDA, NRI Grants with L. Gasbarre and A. Canals on T lymphocyte responses to *Ostertagia* in genetically defined cattle. 1994-1997.
CRADA with Mallinckrodt Veterinary with L. Gasbarre on uses of bovine cytokines in controlling infectious diseases. 1996-1999.

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Peer Reviewed Publications (1993-present):

Christensen, C., Zarlenga, D.S. and Gasbarre, L.C. 1993. *Ostertagia, Haemonchus, Cooperia, Oesophagostomum*: Construction and characterization of genus-specific DNA probes to differentiate important parasites of cattle. Experimental Parasitology. 78:93-100.

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Zarlenga, D.S., Aschenbrenne, R. and Lichtenfels, J.R. 1996. Variations in microsatellite sequences provide evidence for population differences and multiple ribosomal gene repeats within *Trichinella pseudospiralis*. Journal of Parasitology. (In Press).

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